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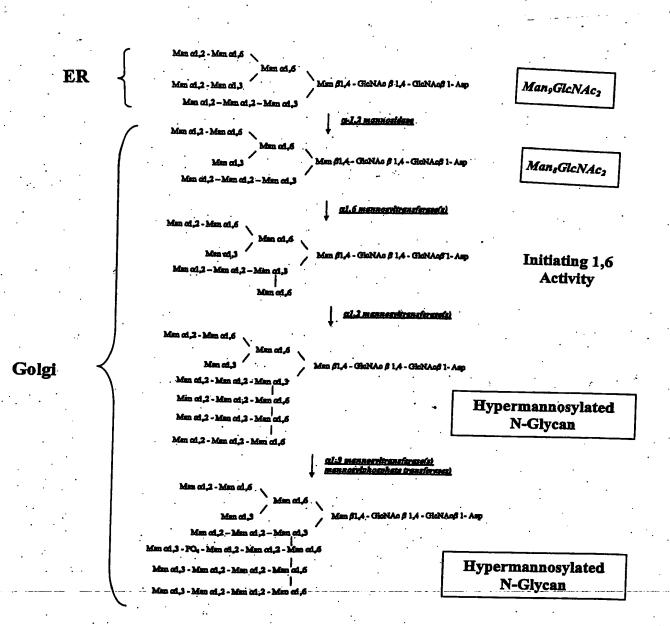


Fig. 1A

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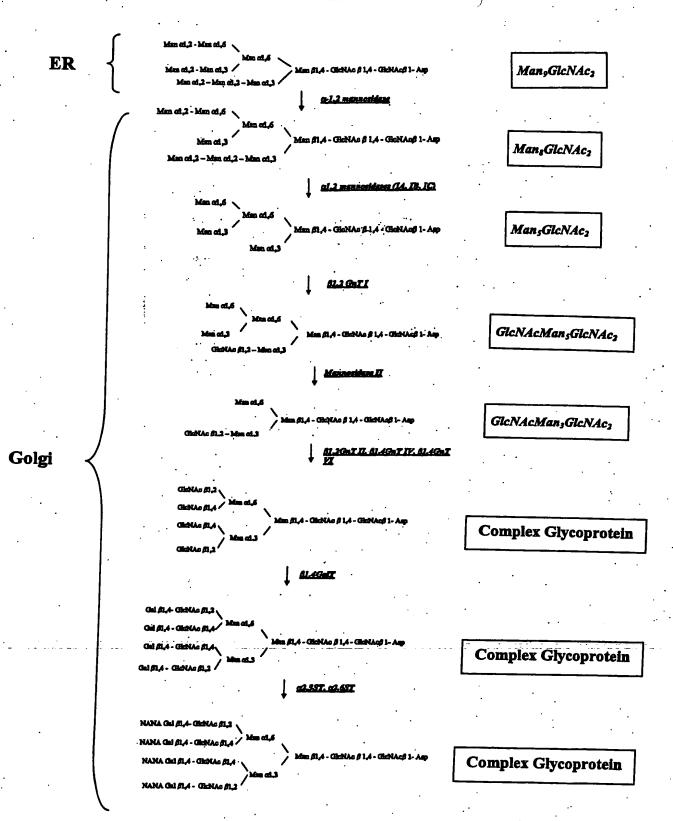
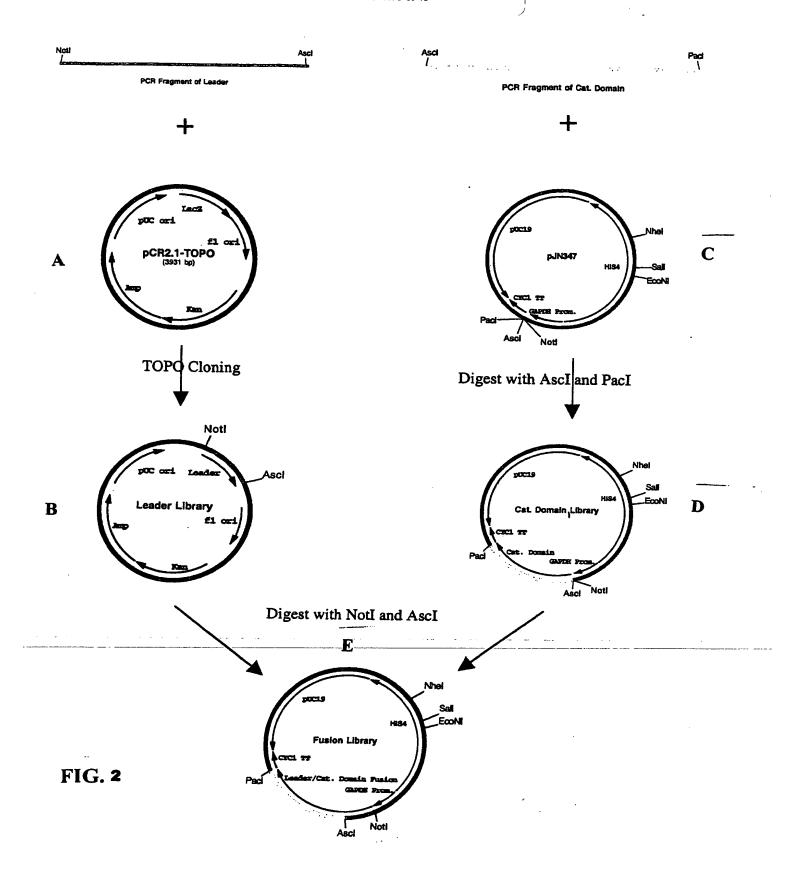


Fig. 1B

10

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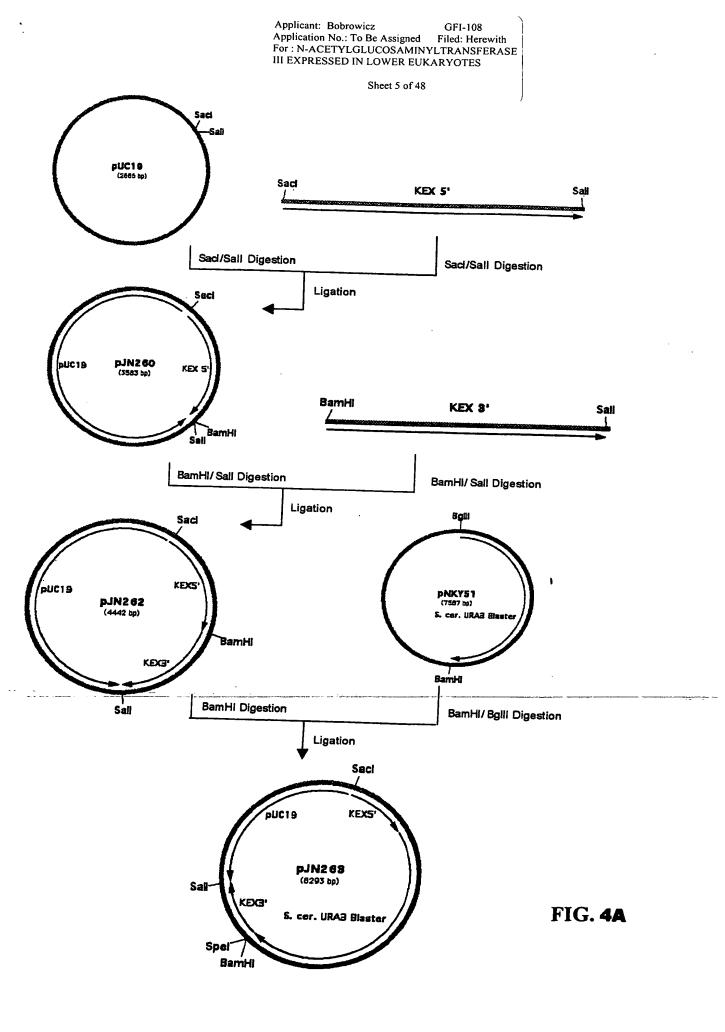


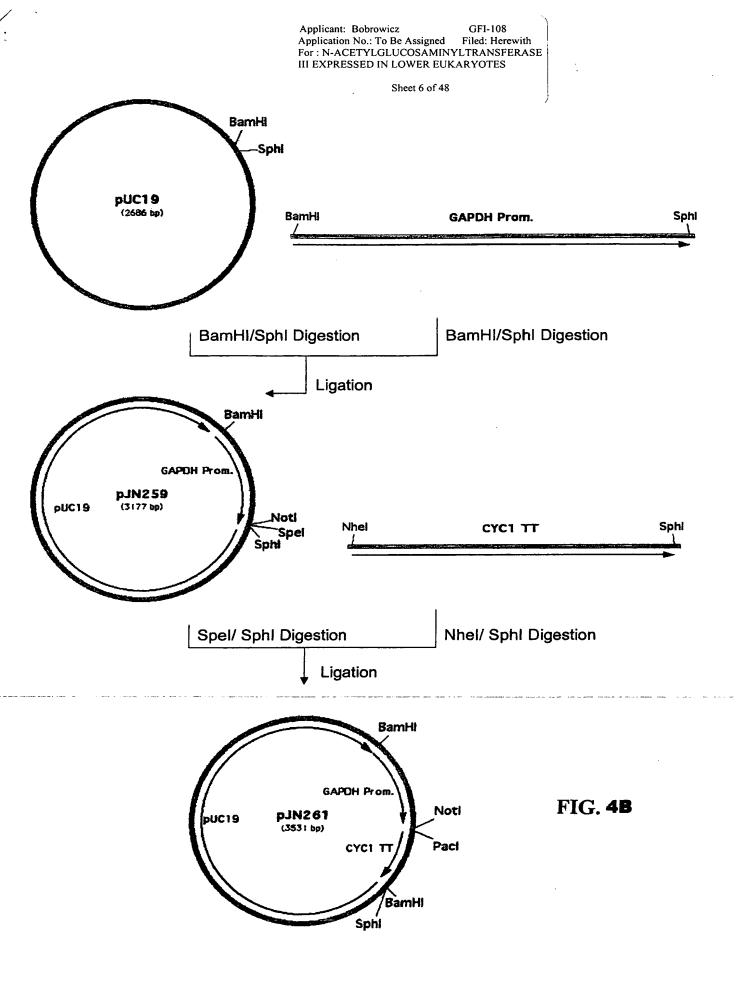
Sheet 4 of 48

FIG. 3

M. musculus alpha-1,2-mannosidase IA open reading frame. The transmembrane and catalytic domains are highlighted in bold respectively. The sequence of the primers used to generate the N-terminal truncations are highlighted by underlining and the start of each respective protein fragment indicated by an arrow.

1 M P V G G L L P L F S S P G G G G G G G G G G G G G R K G 97 totggccccgctgccttccgcctcaccgagaagttcgtgctgctgctgctgcttcagcgccttcatcacgctctgcttcgggggcaatc
33 S G P A A F R L T E K F V L L L V F S A F I T L C F G A I 184 ttetteetgeetgaetcctccaagetgetcagegggteetgttccactccaaccetgeettgcagecggeggagcacaageecgggeteg FLP DSSKLLSGVLFHSNPALQPPAEHKPGL d65 primer 278 gggcgcgtgcggaggatgccgccgaggggagagtccgg<u>caccqcgaggaagqcqcctggggaccctgggagetgg</u>aatggaagacaacttagcca 93 G A R A E D A A E G R V R H R E E G A P G D P G A G L E D N L A
d105 primer 374 ggatccgcgaaaaccacgagcgggctctcagggaagccaaggagaccctgcagaagctgccggaggagatccaaagagacattctgctggagaagg 125 PR I RENHERALREAKETLQKLPEEIQRDILLEK 470 aaaaggtggcccaggaccagctgcgtgacaaggatctgtttaggggcttgcccaaggtggacttcctgcccccgtcgggggtagagaaccgggggg 157 FEKVAQDQLRDKDLFRGLPKVDFLPPVG VENR d187 primer GVKL 1557 gaagttgggad 519 K L G 1639 cggcccgaggtcatcgagacatacatgtac D P 1721 tggaggctctagaaagtcac saggettacgggatgtttacattgcccgtgagagttatg 574 V E A L E S H C R V N Ggatgtccagcaaagtttcttcctggcagagacactgaagtatttgtacttgatattttccgatgatgaccttcttccacta
DVQQSFFLAETLKYLYLIFSDDDLLPL aggeteateettteeetataeteegtgaacagaaggaaattgatggeaaagagaaatga EAHPFPILREQKKEIDGKEK



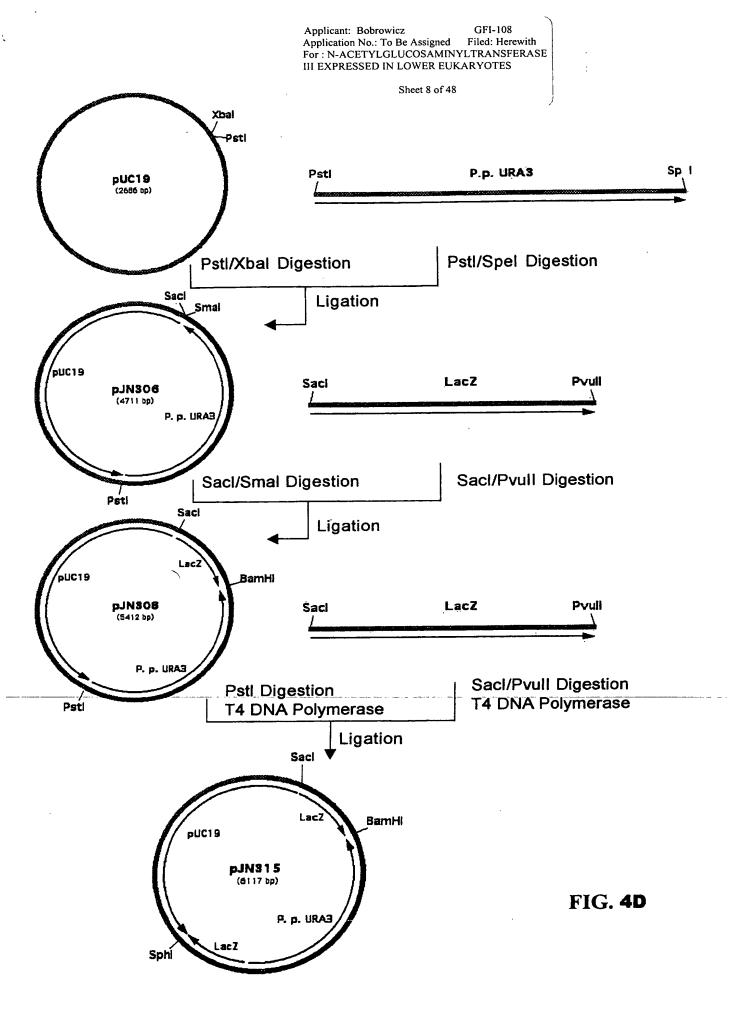


For: N-ACETYLGLUCOSAMINYLTRANSFERASE III EXPRESSED IN LOWER EUKARYOTES Sheet 7 of 48 Saci **PJN269** (8293 bp) Spel OCH 5° Sall. Sall cer. URA3 Blaster Sall/Spel Digestion Sall/Spel Digestion EcoRI Ligation Pmel pJN284 (10324 bp) OCH 3' EcoR Pmel S. cer. URAB Blaste Pmel/EcoRl Digestion Pmel/EcoRl Digestion Spel BamHl Ligation EcoRI **Pmel E H30 89**2NLq (10417 bp). FIG. 4C S. cer. URA3 Bisater Spel BamH

Applicant: Bobrowicz

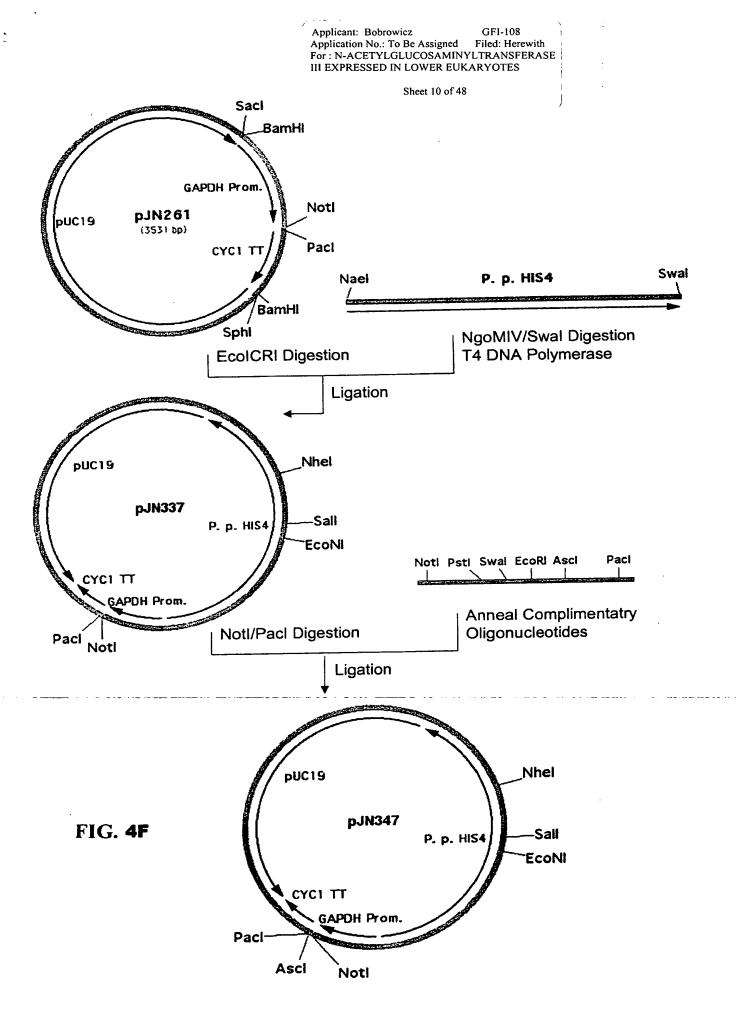
Application No.: To Be Assigned

Filed: Herewith

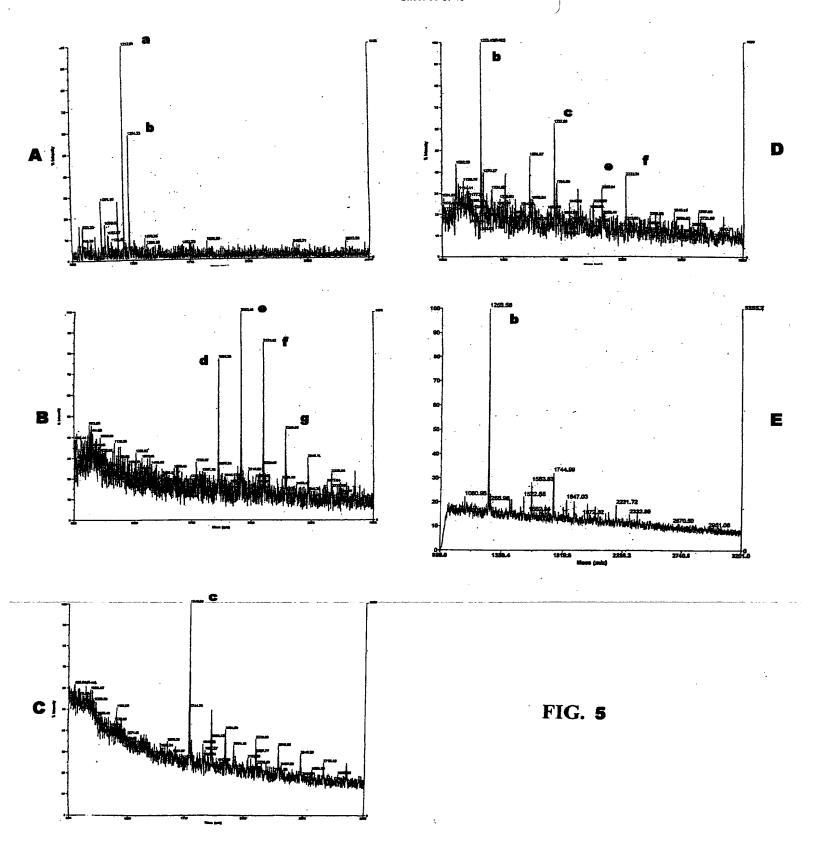


For: N-ACETYLGLUCOSAMINYLTRANSFERASE III EXPRESSED IN LOWER EUKARYOTES Sheet 9 of 48 **EcoRI** BamHi Pmel E HOO **GAPOH Prom.** Noti **pJN261 PJN298** pUC19 (3531 bp) (10417 bp) Pact CYC1 TT S. cer. URA3 Blaster OCH 5' BamHi **BamHI Digestion BamHI Digestion** Sacl EcoRi Ligation OCH 3' **BamHi** pUC19 PIN299 **pJN315** (11260 bp) S. cer. URAS Blaster (6117 bp) OCH 5' P. p. URAS APDH Prom. CYC1 T Sphi BamHi Noti Paci Affil Sacl/Sphl Digestion Pmel/AfIII Digestion **T4 DNA Polymerase** T4 DNA Polymerase Ligation P. p. URAS FIG. 4E pUC19 pJN8 29 (10882 bp) CYC1 TT **GAPDH Prom**

Applicant: Bobrowicz Application No.: To Be Assigned GFI-108 Filed: Herewith

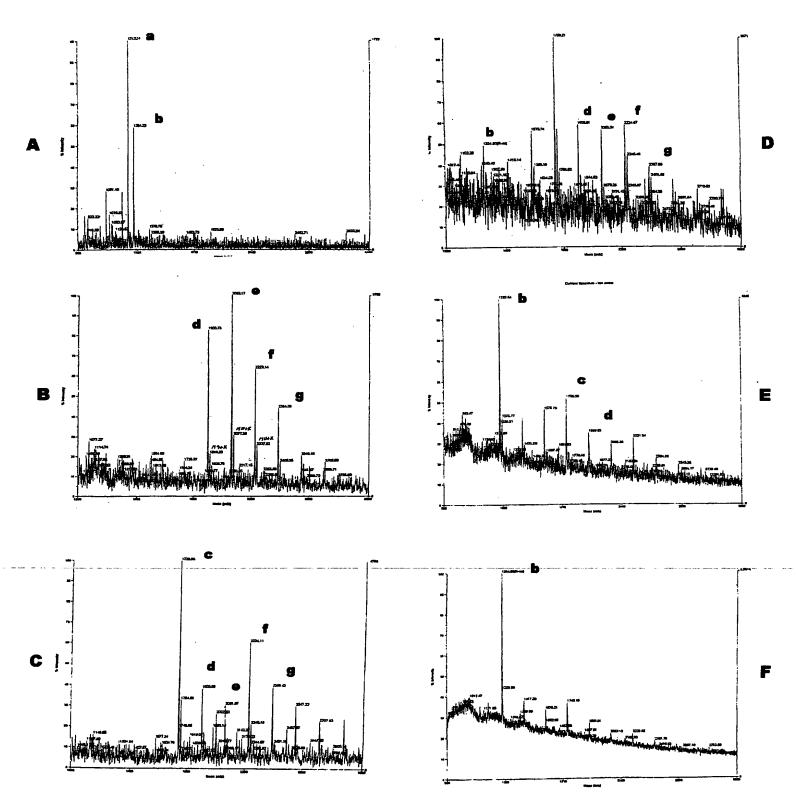


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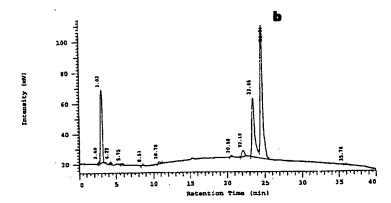


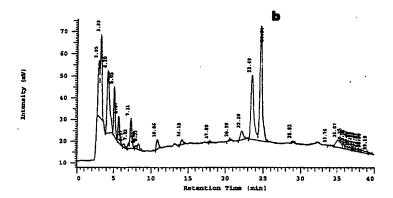
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FIG. 6



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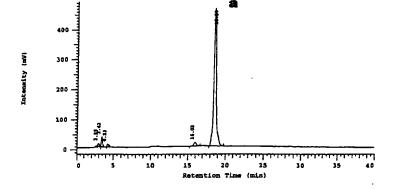


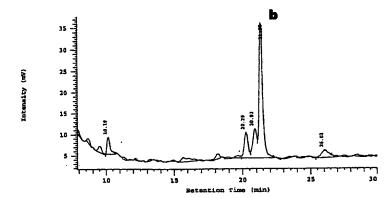
FIG. 7

C

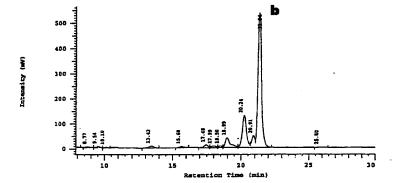
B

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A



B



 \mathbf{C}

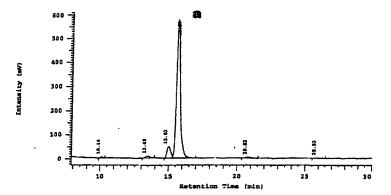
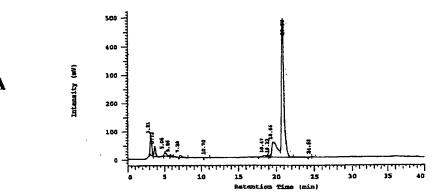
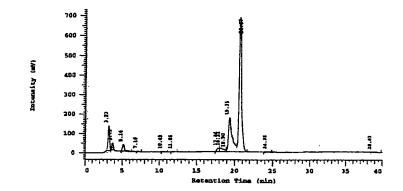


FIG. 8

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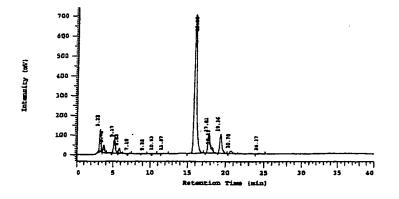


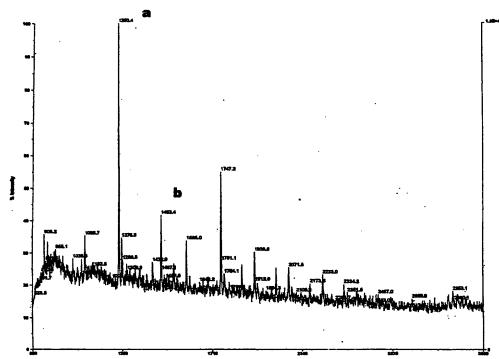
FIG. 9

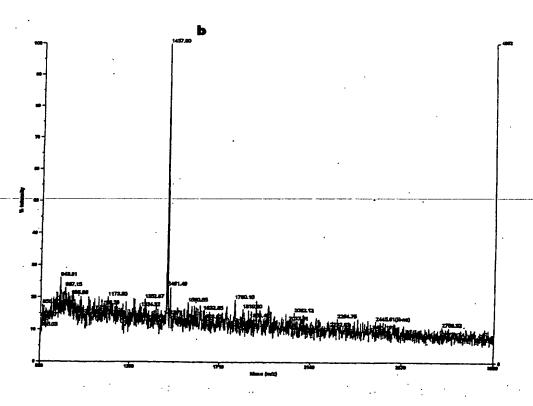
C

B

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В

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pH ptimum f r BB27-2 medium C. elegans Mann1B D31 and S. Mnn10-s

| pН | % f Man5 |
|------|----------|
| 4.5 | 0 |
| 5 | 32 |
| 5.5 | 41 |
| 6 | 35 |
| 6.43 | 22 |
| 6.5 | 4 |
| 7.5 | 0 |
| 8.5 | 0 |

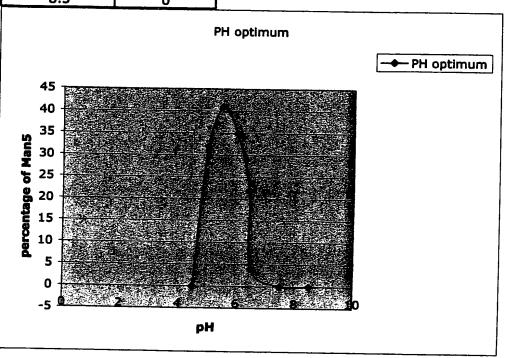


FIG._11_

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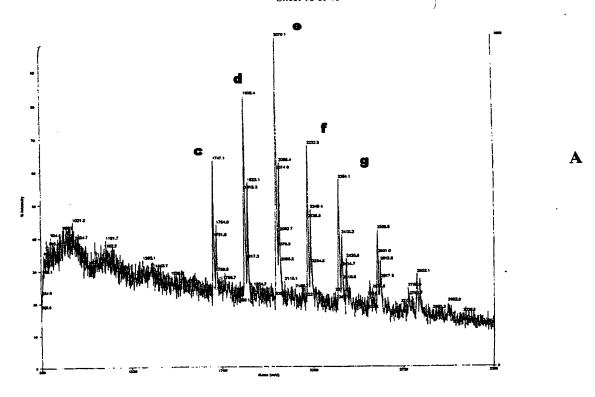
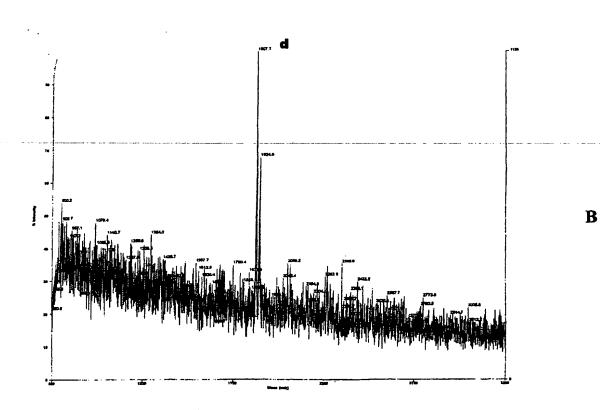


FIG. 12



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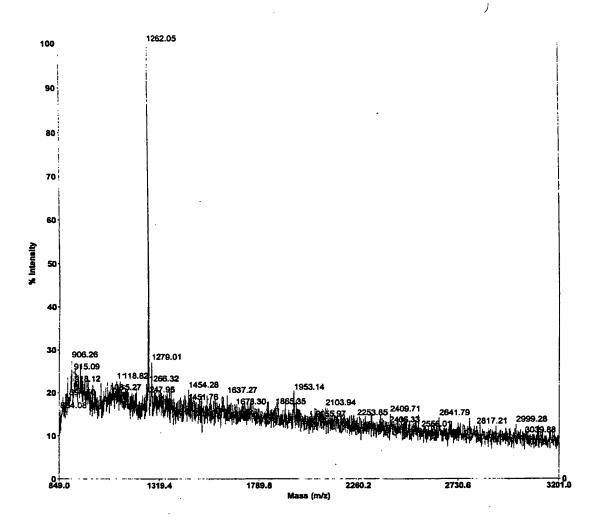


FIG. 12C

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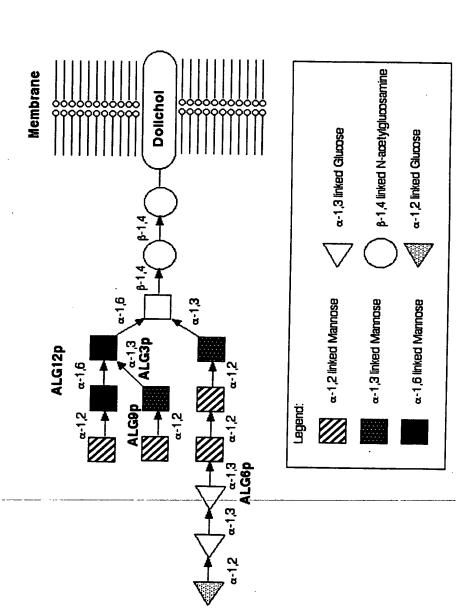


FIG. 13



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Lipid-link d N-glycans

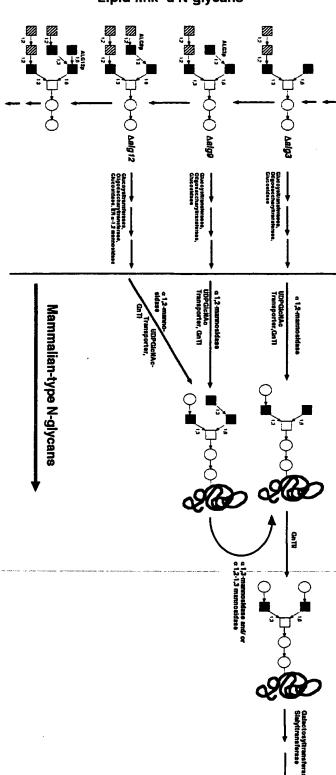


FIG. 14

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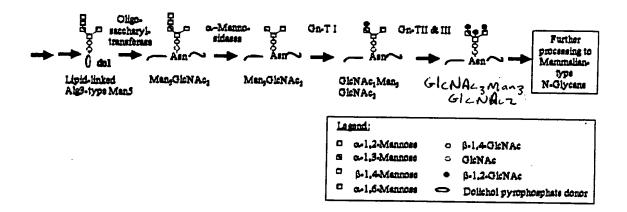


FIG. 15

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ALG3 Blast 05-22-01

| | Sequen | ces | producing significant al | ignments: | (bits) | Value |
|--|--|--------------------------------------|---|--|--|---|
| | gi 302 gi 302 gi 302 gi 107 gi 170 | 4226 4221 4222 2015 7982 | sp Q92685 ALG3_HUMAN D sp Q24332 NT56_DROVI L sp Q27333 NT56_DROME L 3 sp P82149 NT53_DROME sp P40989 GLS2_YEAST 1 | LICHYL-P-MAN:MAN(5)GLO OLICHYL-P-MAN:MAN(5)GLO ETHAL(2)NEIGHBOUR OF C ETHAL(2)NEIGHBOUR OF C LETHAL(2)NEIGHBOUR OF C ,3-BETA-GLUCAN SYNTHAS ,3-BETA-GLUCAN SYNTHAS | LCNAC173 FID P145 FID P121 TID121 SE CO 32 | 0.0 7e-43 3e-34 3e-27 5e-27 2.8 6.6 |
| | Alignm | ents | | | | |
| | Yeast | | | | | |
| >gi 586444 sp P38179 ALG3_YEAST DOLICHYL-P- MAN:MAN(5)GLCNAC(2)-PP-DOLICHYL MANNOSYLTRANSFERASE (DOL-P-MAN DEPENDENT ALPHA(1-3)-MANNOSYLTRANSFERASE) (HM-1 KILLER TOXIN RESISTANCE PROTEIN) Length = 458 | | | | | | |
| | | | 797 bits (2059), Expect s = 422/458 (92%), Posit | | | |
| | Query: Sbjct: | | MEGEQSPQGEKSLQRKQFVRPPLI MEGEQSPQGEKSLQRKQFVRPPLI MEGEQSPQGEKSLQRKQFVRPPLI | DLWQDLKDGVRYVIFDCRANL) | [VMPLLILFESML | CKI |
| | Query: Sbjct: | | IIKKVAYTEIDYKAYMEQIEMIQI IIKKVAYTEIDYKAYMEQIEMIQI IIKKVAYTEIDYKAYMEQIEMIQI | LDGMLDYSQVSGGTGPLVYPAC | SHVLIYKMMYWLT | EGM |
| | - | | DHVERGQVFFRYLYLLTLALQMA DHVERGQVFFRYLYLLTLALQMA | CYYLLHLPPWCVVLACLSKRLF | SIYVLRLFNDCF | TTL |
| | | | DHVERGQVFFRYLYLLTLALQMA | | | |
| | | | FMVVTVLGAIVASRCHQRPKLKKS FMVVTVLGAIVASRCHQRPKLKKS FMVVTVLGAIVASRCHQRPKLKKS | SLALVISATYSMAVSIKMNALI | YFPAMMISLFIL | NDA |
| - | Query: | 241 | NVILTLLDLVAMIAWQVAVAVPFI | LRSFPQQYLHCAFNFGRKFMYQ |)WSINWQMMDEEA | FND 300 |
| | Sbjct: | 241 | NVILTLLDLVAMIAWQVAVAVPFI | LRSFPQQYLHCAFNFGRKFMYQ LRSFPQQYLHCAFNFGRKFMYQ | WSINWQMMDEEA WSINWQMMDEEA | .FND .FND 300 |
| | Query: | 301 | KRFXXXXXXXXXXXXXXXFVTRYI | | | |
| | Sbjct: | 301 | KRF FVTRYI KRFHLALLISHLIALTTLFVTRYI | PRILPDLWSSLCHPLRKNAVLN PRILPDLWSSLCHPLRKNAVLN | ANPAKTIPFVLI ANPAKTIPFVLI | ASN 360 |
| | | | FIGVLFSRSLHYQFLSWYHWTLP1 FIGVLFSRSLHYQFLSWYHWTLP1 | LIFWSGMPFFVGPIWYVLHEW | CWNSYPPNSQ | |
| | Sbjct: | 361 | FIGVLFSRSLHYQFLSWYHWTLP | LLIFWSGMPFFVGPIWYVLHEW | CWNSYPPNSQAS | TLL 420 |
| | | | | KSHLRTTSSMEKKLN | | |
| | Shict. | 421 | T.AT.MTVT.T.T.T.AT.TOT.COCVAT.AL | COULD TO COME VICE NO A CO | | |

Sbjct: 421 LALNTVLLLLLALTQLSGSVALAKSHLRTTSSMEKKLN 458

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Human

>gi|3024226|sp|Q92685|ALG3_HUMAN DOLICHYL-P-MAN:MAN(5)GLCNAC(2)-PP-DOLICHYL MANNOSYLTRANSFERASE (DOL-P-MAN DEPENDENT ALPHA(1-3)-MANNOSYLTRANSFERASE) (NOT56-LIKE PROTEIN) Length = 438Score = 173 bits (439), Expect = 7e-43 Identities = 133/396 (33%), Positives = 195/396 (48%), Gaps = 28/396 (7%) WQDLKDGVRYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLD 85 R ++ + R L+V L L E + +I +VAYTEID+KAYM ++E + ++ Sbjct: 29 WQER----RLLLREPRYTLLVAACLCLAEVGITFWVIHRVAYTEIDWKAYMAEVEGV-IN 83 GMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACY 145 Query: 86 G DY+Q+ G TGPLVYPAG V I+ +Y+ T + Q F LYL TL L Sbjct: 84 GTYDYTQLQGDTGPLVYPAGFVYIFMGLYYATSRGTDIRMAQNIFAVLYLATLLLVFLIY 143 Query: 146 Y-LLHLPPWC-VVLACLSKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKK 203 +PP+ + C S R+HSI+VLRLFND + + +L + OR Sbjct: 144 HQTCKVPPFVFFFMCCASYRVHSIFVLRLFNDP----VAMVLLFLSINLLLAQRWGWG- 197 Query: 204 SLALVISATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVAVPF 263 +S+AVS+KMN LL+ P ++ L L L + A + QV + +PFSbjct: 198 -----CCFFSLAVSVKMNVLLFAPGLLFLLLTQFGFRGALPKLGICAGL--QVVLGLPF 249 L P YL +F+ GR+F++ W++NW+ + E F + FSbjct: 250 LLENPSGYLSRSFDLGRQFLFHWTVNWRFLPEALFLHRAFHLALLTAHLTLLLLFALCRW 309 Query: 324 PRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASNFIGVLFSRSLHYQFLSWYHWTLP 383 + S L P ++ I L SNFIG+ FSRSLHYQF WY TLP Sbjct: 310 HRTGESILSLLRDPSKRKVPPQPLTPNQIVSTLFTSNFIGICFSRSLHYQFYVWYFHTLP 369

Drosophila Vi

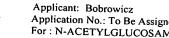
>gi|3024221|sp|Q24332|NT56_DROVI LETHAL(2)NEIGHBOUR OF TID PROTEIN (NOT58)
Length = 526

Query: 384 ILIF----WSGMPFFVGPIWYVLHEWCWNSYPPNS 414

Sbjct: 370 YLLWAMPARWLTHLLRLLVLGLI--ELSWNTYPSTS 403

L++ W + + + E WN+YP S

```
Score = 145 bits (366), Expect = 3e-34
 Identities = 103/273 (37%), Positives = 157/273 (56%), Gaps = 17/273 (6%)
Query: 33 VRYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQ 92
          ++Y+ F+ A IV L++L E+++ ++I++V YTEID+KAYM++ E L+G +YS
Sbjct: 34 IKYLAFEPAALPIVSVLIVLAEAVINVLVIQRVPYTEIDWKAYMQECEGF-LNGTTNYSL 92
Query: 93 VSGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACYYLLH-LP 151
          + G TGPLVYPA V IY +Y+LT
                                   +V Q F +YLL + L + Y
Sbjct: 93 LRGDTGPLVYPAAFVYIYSGLYYLTGQGTNVRLAQYIFACIYLLQMCLVLRLYTKSRKVP 152
Query: 152 PWCVVLACL-SKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKKSLALVIS 210
                  S R+HSIYVLRLFND L +L A +
          P+ +VL+
                                                    QR L
Sbjct: 153 PYVLVLSAFTSYRIHSIYVLRLFNDPVAIL----LLYAALNLFLDQRWTLG-----S 200
Query: 211 ATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVAVPFLRSFPQQ 270
            YS+AV+KMN+A+LFL+V+TL+L
                                                    Q+ + PFLR+ P +
Sbjct: 201 ICYSLAVGVKMN--ILLFAPALLLFYLANLGVLRTLVQLTICAVLQLFIGAPFLRTHPME 258
```



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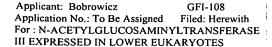
Query: 271 YLHCAFNFGRKFMYQWSINWQMMDEEAFNDKRF 303 YL +F+ GR F ++W++N++ + +E F + F Sbjct: 259 YLRGSFDLGRIFEHKWTVNYRFLSKELFEQREF 291 Score = 53.3 bits (127), Expect = 1e-06 Identities = 31/62 (50%), Positives = 41/62 (66%), Gaps = 6/62 (9%) Query: 352 IPFVLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLH--EWCWNS 409 +PF L NFIGV +RSLHYQF WY +LP L+ WS P+ +G + +L E+CWN+ Sbjct: 412 LPFFL--CNFIGVACARSLHYQFYIWYFHSLPYLV-WS-TPYSLGVRYLILGIIEYCWNT 467 Query: 410 YP 411 Sbjct: 468 YP 469 Drosophila melanogaster >qi|3024222|sp|Q27333|NT56 DROME LETHAL(2)NEIGHBOUR OF TID PROTEIN (NOT56) (NOT45) Length = 510 Score = 121 bits (305), Expect = 3e-27 Identities = 96/272 (35%), Positives = 154/272 (56%), Gaps = 17/272 (6%) Query: 34 RYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQV 93 +Y++ + A IV ++L E ++ ++I++V YTEID+ AYM++ E Sbjct: 36 KYLLLEPAALPIVGLFVLLAELVINVVVIQRVPYTEIDWVAYMQECEGF-LNGTTNYSLL 94 Query: 94 SGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACYYLLH-LPP 152 G TGPLVYPA V IY +Y++T +V Q F +YLL LAL + Y +PP Sbjct: 95 RGDTGPLVYPAAFVYIYSALYYVTSHGTNVRLAQYIFAGIYLLQLALVLRLYSKSRKVPP 154 Query: 153 WCVVLACL-SKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKKSLALVISA 211 + +VL+ S R+HSIYVLRLFND + V +L A + +R L Sbjct: 155 YVLVLSAFTSYRIHSIYVLRLFNDP-----VAVLLLYAALNLFLDRRWTLG------ST 202 Query: 212 TYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVAVPFLRSFPQQY 271 +S+AV + KMN + A + LF L + ++ T+L LQ+ + PFL + P +Y Sbjct: 203 FFSLAVGVKMN--ILLFAPALLLFYLANLGLLRTILOLAVCGVIOLLLGAPFLLTHPVEY 260 Query: 272 LHCAFNFGRKFMYQWSINWQMMDEEAFNDKRF 303 L +F+ GR F ++W++N++ + F ++ F Sbjct: 261 LRGSFDLGRIFEHKWTVNYRFLSRDVFENRTF 292

Score = 49.4 bits (117), Expect = 2e-05Identities = 27/60 (45%), Positives = 35/60 (58%), Gaps = 2/60 (3%)

Query: 352 IPFVLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYP 411 +PF L N +GV SRSLHYQF WY +LP L + + V + L E+CWN+YP Sbjct: 407 LPFFL--CNLVGVACSRSLHYQFYVWYFHSLPYLAWSTPYSLGVRCLILGLIEYCWNTYP 464

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Matrix: BLOSUM62 Gap Penalties: Existence: 11, Extension: 1 Number of Hits to DB: 28883317 Number of Sequences: 96469 Number of extensions: 1107545 Number of successful extensions: 2870 Number of sequences better than 10.0: 16 Number of HSP's better than 10.0 without gapping: 5 Number of HSP's successfully gapped in prelim test: 11 Number of HSP's that attempted gapping in prelim test: 2839 Number of HSP's gapped (non-prelim): 23 length of query: 458
length of database: 35,174,128 effective HSP length: 45 effective length of query: 413 effective length of database: 30,833,023 effective search space: 12734038499 effective search space used: 12734038499 T: 11 A: 40 X1: 15 (7.1 bits) X2: 38 (14.6 bits) X3: 64 (24.7 bits) S1: 40 (21.8 bits) S2: 67 (30.4 bits)



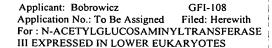
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S. cerevisiae ALG3

ATGGAAGGTGAACAGTCTCCGCAAGGTGAAAAGTCTCTGCAAAGGAAGC AATTTGTCAGACCTCCGCTGGATCTGTGGCAGGATCTCAAGGACGGTGTG CGCTACGTGATCTTCGATTGTAGGGCCAATCTTATCGTTATGCCCCTTTTG ATTTTGTTCGAAAGCATGCTGTGCAAGATTATCATTAAGAAGGTAGCTTAC ACAGAGATCGATTACAAGGCGTACATGGAGCAGATCGAGATGATTCAGCT CGATGCCATGCTGGACTACTCTCAGGTGAGTGGTGGAACGGGCCCGCTGG TGTATCCAGCAGGCCACGTCTTGATCTACAAGATGATGTACTGGCTAACA GAGGGAATGGACCACGTTGAGCGCGGGCAAGTGTTTTTCAGATACTTGTA TCTCCTTACACTGGCGTTACAAATGGCGTGTTACTACCTTTTACATCTACC ACCGTGGTGTGTGGTCTTGGCGTGCCTCTCTAAAAGATTGCACTCTATTTA CGTGCTACGGTTATTCAATGATTGCTTCACTACTTTGTTTATGGTCGTCACG GTTTTGGGGGCTATCGTGGCCAGCAGGTGCCATCAGCGCCCCAAATTAAA GAAGTCCCTTGCGCTGGTGATCTCCGCAACATACAGTATGGCTGTGAGCA TTAAGATGAATGCGCTGTTGTATTTCCCTGCAATGATGATTTCTCTATTCAT CCTTAATGACGCGAACGTAATCCTTACTTTGTTGGATCTCGTTGCGATGAT TGCATGGCAAGTCGCAGTTGCAGTGCCCTTCCTGCGCAGCTTTCCGCAACA GTACCTGCATTGCGCTTTTAATTTCGGCAGGAAGTTTATGTACCAATGGAG TATCAATTGGCAAATGATGGATGAAGAGGCTTTCAATGATAAGAGGTTCC ACTTGGCCCTTTTAATCAGCCACCTGATAGCGCTCACCACACTGTTCGTCA CAAGATACCCTCGCATCCTGCCCGATTTATGGTCTTCCCTGTGCCATCCGC TGAGGAAAAATGCAGTGCTCAATGCCAATCCCGCCAAGACTATTCCATTC GTTCTAATCGCATCCAACTTCATCGGCGTCCTATTTTCAAGGTCCCTCCAC TACCAGTTTCTATCCTGGTATCACTGGACTTTGCCTATACTGATCTTTTGGT CGGGAATGCCCTTCTTCGTTGGTCCCATTTGGTACGTCTTGCACGAGTGGT GCTGGAATTCCTATCCACCAAACTCACAAGCAAGCACGCTATTGTTGGCA TTGAATACTGTTCTGTTGCTTCTATTGGCCTTGACGCAGCTATCTGGTTCGG TCGCCCTCGCCAAAAGCCATCTTCGTACCACCAGCTCTATGGAAAAAAAG **CTCAACTGA**

S. cerevisiae Alg3p

MEGEQSPQGEKSLQRKQFVRPPLDLWQDLKDGVRYVIFDCRANLIVMPLLIL FESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQVSGGTGPLVYPAG HVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACYYLLHLPPWCV VLACLSKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKKSLALV ISATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVA VPFLRSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFNDKRFHLALLISHL IALTTLFVTRYPRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASNFIGVLFS RSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYPPNSQASTL LLALNTVLLLLALTQLSGSVALAKSHLRTTSSMEKKLN



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P. pastoris ALG3 ATGCCTCCGATAGAGCCAGCTGAAAGGCCAAAGCTTACGCTGAAAAATGT AGTGTCTTCGTTGCACCTCTTTTATGGTTAGCTGATTCCATTGTTATCAAGG TGATCATTGGCACTGTTTCCTACACAGATATTGATTTTCTTCATATATGCA ACAAATCTTTAAAATTCGACAAGGAGAATTAGATTATAGCAACATATTTG GTGACACCGGTCCATTGGTTTACCCAGCCGGCCATGTTCATGCTTACTCAG TACTTTCGTGGTACAGTGATGGTGGAGAAGACGTCAGTTTCGTTCAACAA GCATTTGGTTGGTTATACCTAGGTTGCTTGTTACTATCCATCAGCTCCTACT GTCCAAGAGACTGCATTCAATATTTGTATTGAGACTCTTCAATGACTGTTT CTGGAGGAAAGATGGCACAACTATTCCATTATCTGTCCCTGATGCTGCAG ATACGTACAGTTTAGCCATCTCTGTAAAGATGAATGCGCTGCTATACCTCC CAGCATTCCTACTACTCATATATCTCATTTGTGACGAAAATTTGATTAAAG CCTTGGCACCTGTTCTAGTTTTGATATTGGTGCAAGTAGGAGTCGGTTATT CGTTCATTTTACCGTTGCACTATGATGATCAGGCAAATGAAATTCGTTCTG CCTACTTTAGACAGGCTTTTGACTTTAGTCGCCAATTTCTTTATAAGTGGA CGGTTAATTGGCGCTTTTTGAGCCAAGAAACTTTCAACAATGTCCATTTTC ACCAGCTCCTGTTTGCTCTCCATATTATTACGTTAGTCTTGTTCATCCTCAA GTTCCTCTCTCAAAAACATTGGAAAACCGCTTGGTAGATTTGTGTTGGA CATTTTCAAATTTTGGAAGCCAACCTTATCTCCAACCAATATTATCAACGA CCCAGAAAGAAGCCCAGATTTTGTTTACACCGTCATGGCTACTACCAACTT AATAGGGGTGCTTTTTGCAAGATCTTTACACTACCAGTTCCTAAGCTGGTA TGCGTTCTCTTTGCCATATCTCCTTTACAAGGCTCGTCTGAACTTTATAGCA CAGAACAAAGTTCCGCGTTGTTGGTATCTATCTTACTACTTATCCTGATTC TCATTTTTACCAACGAACAGTTATTTCCTTCTCAATCGGTCCCTGCAGAAA **AAAAGAATACATAA**

P. pastoris Alg3p
MPPIEPAERPKLTLKNVIGDLVALIQNVLFNPDFSVFVAPLLWLADSIVIKVIIG
TVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYPAGHVHAYSVLSWYS
DGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSIF
VLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVKMN
ALLYLPAFLLLIYLICDENLIKALAPVLVLILVQVGVGYSFILPLHYDDQANEIR
SAYFRQAFDFSRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFILKF
LSPKNIGKPLGRFVLDIFKFWKPTLSPTNIINDPERSPDFVYTVMATTNLIGVLF
ARSLHYQFLSWYAFSLPYLLYKARLNFIASIIVYAAHEYCWLVFPATEQSSAL
LVSILLLILILIFTNEQLFPSQSVPAEKKNT

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P. pastoris ALG3 BLAST

| sequences | producing significant alignments: | (bits) | Value |
|--|---|---|--|
| gi 586444 gi 128023 gi 984725 gi 749270 gi 162265 gi 253672 gi 258147 gi 175350 gi 165400 gi 132792 gi 221223 gi 212920 | sp P38179 ALG3 YEAST Dolichyl-P-Man:Man(5)GlcNAc(65 gb AAK07848.1 AF309689 10 putative NOT-56 manno gb AAA75352.1 ORF 1 2 pir T39084 probable mannosyltransferase - fissi 31 gb AAL16193.1 AF428424 1 | .228 .212 206 .176 .164 .161 .160 .155 .154 | Value 2e-58 8e-54 4e-52 8e-43 2e-39 2e-38 3e-38 2e-36 2e-36 3e-35 4e-26 3e-24 |
| g | | | |
| S. cerevia Score = Identitie | siae 228 bits (580), Expect = 2e-58 es = 154/429 (35%), Positives = 229/429 (53%), Gaps = | 37/429 | (8%) |
| Query: 9 Sbjct: 20 | RPKLTLKNVIGDLVALIQNVLFNPDFSVFVAPLLWLADSIVIKVIIGTVSYT RP L L DL ++ V+F+ ++ V PLL L +S++ K+II V+YT RPPLDLWQDLKDGVRYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYT | TATDA A | VM |
| | QQIFKIR-QGELDYSNIFGDTGPLVYPAGHVHAYSVLSWYSDGGEDVSFVQQ +QI I+ G LDYS + G TGPLVYPAGHV Y ++ W ++G + V Q EQIEMIQLDGMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQV | E AT.V | т. |
| Query: 128 | CLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSIFVLRLFNDCLTTFLMLAT | 'III | LQ 184 |
| | QASSWRKDGTTIPLSVPDAADTYSLAISVKMNXXXXXXXXXXXXXXXCDENL + K ++ L + + TYS+A+S+KMN D N+ RCHQRPKLKKSLALVISATYSMAVSIKMNALLYFPAMMISLFILNDANV | т т. | |
| Query: 245 | XXXXXXXXXYSFILPLHYDDQANEIRSAYFRQAFDFSRQFLYKWTVNWRFL F+ Y AF+F R+F+Y+W++NW+ + AMIAWQVAVAVPFLRSFPQQYLHCAFNFGRKFMYQWSINWQMM | SQETFN | TV 304 |
| | | | |
| | HFHQLLFALHIITL-VLFILKFLSPKNIGKPLGRFVLDIFKFWKPTLSPTNI FH L H+I L LF+ ++ R + D++ L + | ±M ±D . | |
| | RFHLALLISHLIALTTLFVTRYPRILPDLWSSLCHPLRKNAV | | |
| | PDFVYTVMATTNLIGVLFARSLHYQFLSWYAFSLPYLLYKARLNFIASIIVY | UF.CW | 1 |
| Sbjct: 352 | IPFVLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWY | VLHEWCW | N 408 |
| Query: 423 | VFPATEQSS 431 +P Q+S | | |
| Sbjct: 409 | SYPPNSQAS 417 | | |

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Neurospora crassa

| Identities | 12 bits (540), Expect = 8e-54 12 = 140/400 (35%), Positives = 212/400 (53%), Gaps = 29/400 (7%) | | |
|---------------------------|---|--|--|
| | SVFVAPLLWLADSIVIKVIIGTVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYP 94 S + P L+L D+++ +II V YT+ID+++YM+Q+ +I GE DY+ + G TGPLVYP | | |
| _ | SKLIPPALFLVDALLCGLIIWKVPYTEIDWAAYMEQVSQILSGERDYTKVRGGTGPLVYP 92 | | |
| | AGHVHAYSVLSWYSDGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVA 154 A HV+ Y+ L +D G ++ QQ F LY+ L + + Y+ K PP F LL | | |
| | AAHVYIYTGLYHLTDEGRNILLAQQLFAGLYMVTLAVVMGCYWQAKAPPYLFPLLTL 149 | | |
| Query: 15 | SKRLHSIFVLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVK 214 SKRLHSIFVLR FNDC + I Q+ +W+ A Y+L + VK SKRLHSIFVLRCFNDCFAVLFLWLAIFFFQR-RNWQAGALLYTLGLGVK 197 | | |
| | | | |
| = | MNXXXXXXXXXXXXXXXCDENLIKALAPXXXXXXXXXXXXXXXYSFILPLHYDDQANEIRSAY 274 M + + L F+ HY + Y | | |
| = | MTLLLSLPAVGIVLFLGSG-SFVTTLQLVATMGLVQILIGVPFLAHYPTEY 247 | | |
| _ | FRQAFDFSRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFI-LKFLSPKNIGK 333 +AF+ SRQF +KWTVNWRF+ +E F + F L ALH++ L +FI +++ P K | | |
| = | 8 LSRAFELSROFFFKWTVNWRFVGEEIFLSKGFALTLLALHVLVLGIFITTRWIKPARK 305 | | |
| | 4 PLGRFVLDIFKFWKPTLS-PTNIINDPERSPDFVYTVMATTNLIGVLFARSLHYQFLSWY 392 L + + + KP L+ P + + + + + + + + + + + + + + + + + | | |
| Sbjct: 30 | 6 SLVQLISPVLLAGKPPLTVPEHRAAARDVTPRYIMTTILSANAVGLLFARSLHYQFYAYV 365 | | |
| _ - | 3 AFSLPYLLYKARLNFIASIIVYAAHEYCWLVFPATEQSSA 432 A+S P+LL++A L+ + +++A HE+ W VFP+T SSA | | |
| Sbjct: 36 | 6 AWSTPFLLWRAGLHPVLVYLLWAVHEWAWNVFPSTPASSA 405 | | |
| Schizosaccharomyces pombe | | | |
| Score = Identiti | 176 bits (445), Expect = 8e-43 es = 132/390 (33%), Positives = 194/390 (49%), Gaps = 35/390 (8%) | | |
| Query: 42 | LWLADSIVIKVIIGTVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYPAGHVHAY 101 L L + + II V YT+ID+ +YM+Q+ GE DY ++ G TGPLVYP GHV Y | | |
| - | LLLLEIPFVFAIISKVPYTEIDWIAYMEQVNSFLLGERDYKSLVGCTGPLVYPGGHVFLY 89 | | |
| = | 2 SVLSWYSDGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSI 161 ++L + +DGG ++ Q F ++Y + +I Y F + + P +VLL+ SKRLHSI | | |
| Sbjct: 90 | TLLYYLTDGGTNIVRAQYIFAFVYWITTAIVGYLFK-IVRAPFYIYVLLILSKRLHSI 146 | | |
| | 2 FVLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVKMNXXXXX 221 F+LRLFND + L + I+ W + A+ S+A SVKM+ | | |
| | 7 FILRLFNDGFNS-LFSSLFILSSCKKKWVRASILLSVACSVKMSSLLYV 194 | | |
| - | 2 XXXXXXXXXCDENLIKALAPXXXXXXXXXXXXYSFILPLHYDDQANEIRSAYFRQAFDF 281 L++ L P + + + + +Y+ QAFDF | | |
| Sbjct: 19 | 5 PAYLVLLLQILGPKKTWMHIFVIIIVQILFSIPFLAYFWSYWTQAFDF 242 | | |
| · • | 2 SRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFILKFLSPKNIGKPLGRFVLD 341 R F YKWTVNWRF+ + F + F + LH+ LV F K + + P | | |
| - | 3 GRAFDYKWTVNWRFIPRSIFESTSFSTSILFLHVALLVAFTCKHWNKLSRATP 295 | | |
| Query: 34 | 2 IFKFWKPTLSPTNIINDPERSPDFVYTVMATTNLIGVLFARSLHYQFLSWYAFSLPYLLY 401 F L+ + +P+F++T +AT+NLIG+L ARSLHYQF +W+A+ PYL Y | | |

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| Sbjct: 296 -FAMVNSMLTLKPLPKLQLATPNFIFTALATSNLIGILCARSLHYQFYAWFAWYSPYLCY 354 |
|---|
| Query: 402 KARLNFIASIIVYAAHEYCWLVFPATEQSS 431 +A I ++ EY W VFP+T+ SS |
| Sbjct: 355 QASFPAPIVIGLWMLQEYAWNVFPSTKLSS 384 Arabidopsis thaliana |
| Score = 164 bits (415), Expect = 2e-39 Identities = 131/391 (33%), Positives = 194/391 (49%), Gaps = 29/391 (7%) |
| Query: 42 LWLADSIVIKVIIGTVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYPAGHVHAY 101 L LAD+I++ +II V YT ID+ +YM Q+ GE DY N+ GDTGPLVYPAG ++ Y |
| Sbjct: 39 LILADAILVALIIAYVPYTKIDWDAYMSQVSGFLGGERDYGNLKGDTGPLVYPAGFLYVY 98 |
| Query: 102 SVLSWYSDGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSI 161 S + + G +V Q FG LY+ L + + Y + + +P LL SKR+HSI |
| Sbjct: 99 SAVQNLTGGEVYPAQILFGVLYIVNLGIVLIIYVKTDVVPWWALSLLCLSKRIHSI 154 |
| Query: 162 FVLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVKMNXXXXX 221 FVLRLFNDC L+ A++ + +RK + + +S A+SVKMN |
| Sbjct: 155 FVLRLFNDCFAMTLLHASMALFLYRKWHLGMLVFSGAVSVKMNVLLYA 202 |
| Query: 222 XXXXXXXXXCDENLIKALAPXXXXXXXXXXXXXYSFILPLHYDDQANEIRSAYFRQAFDF 281 N+I ++ F++ +Y AFD |
| Sbjct: 203 PTLLLLLLKAMNIIGVVSALAGAALAQILVGLPFLITYPVSYIANAFDL 251 |
| Query: 282 SRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFILKFLSPKNIGKPLGRFVLD 341 R F++ W+VN++F+ + F + F L H+ LV F + K+ G +G |
| Sbjct: 252 GRVFIHFWSVNFKFVPERVFVSKEFAVCLLIAHLFLLVAFA-NYKWCKHEGGIIGFMRSR 310 |
| Query: 342 IFKFWKP-TLSPTNIINDPERSPDFVYTVMATTNLIGVLFARSLHYQFLSWYAFSLPYLL 400 F P +LS +++ + + V T M N IG++FARSLHYOF SWY +SLPYLL |
| Sbjct: 311 HFFLTLPSSLSFSDVSASRIITKEHVVTAMFVGNFIGIVFARSLHYQFYSWYFYSLPYLL 370 |
| Query: 401 YKARLNFIASIIVYAAHEYCWLVFPATEQSS 431 ++ +I++ E CW V+P+T SS |
| Sbjct: 371 WRTPFPTWLRLIMFLGIELCWNVYPSTPSSS 401 |

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K. lactis ALG3

TTTGTTTACAAGCTGATACCAACGAACATGAATACACCGGCAGGTTTACT
GAAGATTGGCAAAGCTAACCTTTTACATCCTTTTACCGATGCTGTATTCAG
TGCGATGAGAGTAAACGCAGAACAAATTGCATACATTTACTTGTTACCA
ATTACATTGGAGTACTATTTGCTCGATCATTACACTACCAATTCCTATCTT
GGTACCATTGGACGTTACCAGTACTATTGAATTGGGCCAATGTTCCGTATC
CGCTATGTGTGCTATGGTACCTAACACATGAGTGGTGCTGGAACAGCTAT
CCGCCAAACGCTACTGCATCCACACTGCTACACGCGTGTAACACATACTG
TTATTGGCTGTATTCTTAAGAGGACCCGCAAACTCGAAAAGTGGTGATAA
CGAAACACACACGAGAAAGCTGAG

K. lactis Alg3p

FVYKLIPTNMNTPAGLLKIGKANLLHPFTDAVFSAMRVNAEQIAYILLVTNYI GVLFARSLHYQFLSWYHWTLPVLLNWANVPYPLCVLWYLTHEWCWNSYPP NATASTLLHACNTYCYWLYSZEDPQTRKVVITKQHTRKL

FIG. 20

Applicant: Bobrowicz Filed: Herewith Application No.: To Be Assigned For: N-ACETYLGLUCOSAMINYLTRANSFERASE III EXPRESSED IN LOWER EUKARYOTES

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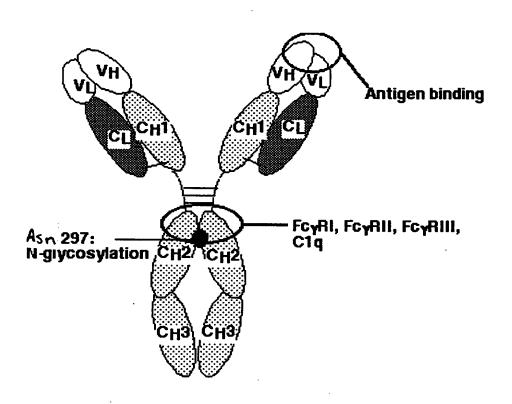


| Score E Sequences producing significant alignments: | (bits) | Value | | |
|---|----------------------|--|--|--|
| qi 586444 sp P38179 ALG3 YEAST Dolichyl-P-Man:Man(5)GlcNAc(. qi 984725 gb AAA75352.1 ORF 1 qi 16226531 gb AAL16193.1 AF428424 1 At2g47760/F17A22.15 [A.gi 25367230 pir B84919 Not56-like protein [imported] - Ara.gi 21292031 gb EAA04176.1 agCP3388 [Anopheles gambiae strgi 20892051 ref XP 148657.1 similar to Lethal(2)neighbour | 72 72 69 | 1e-28 4e-19 1e-12 1e-12 2e-11 2e-10 | | |
| Alignments | | | | |
| S. cerevisiae | | | | |
| Score = 125 bits (314), Expect = 1e-28 Identities = 60/120 (50%), Positives = 83/120 (69%), Gaps = 1/120 (0%) Frame = +3 | | | | |
| Query: 66 ANLLHPFT-DAVFSAMRVNAEQIAYILLVTNYIGVLFARSLHYQFLSWYI | HWTLPVLL HWTLP+L+ | NWA 242 W+ | | |
| Sbjct: 332 SSLCHPLRKNAVLNANPAKTIPFVLIASNFIGVLFSRSLHYQFLSWY | HWTLPILI | FWS 389 | | |
| | V + K | HK | | |
| Sbjct: 390 GMPFFVGPIWYVLHEWCWNSYPPNSQASTLLLALNTVLLLLLA-LTQLS | GSVALAKS | HLR 448 | | |

A. thaliana

```
Score = 72.0 bits (175), Expect = 1e-12
Identities = 42/107 (39%), Positives = 57/107 (53%), Gaps = 3/107 (2%)
 Frame = +3
Query: 84 FTDAVFSAMRVNAEQIAYILLVTNYIGVLFARSLHYQFLSWYHWTLPVLLNWANVPYPLC 263
          F+D S + + E + + V N+IG++FARSLHYQF SWY ++LP LL
Sbjct: 322 FSDVSASRI-ITKEHVVTAMFVGNFIGIVFARSLHYQFYSWYFYSLPYLLWRTPFPTWLR 380
Query: 264 VLWYLTHEWCWNSYPPNATASTL---LHACNTYCYWLYS*EDPQTRK 395
          ++ +L E CWN YP
                           ++S L LH
                                             WL
                                                  DP
                                                       K
Sbjct: 381 LIMFLGIELCWNVYPSTPSSSGLLLCLHLIILVGLWLAPSVDPYQLK 427
```

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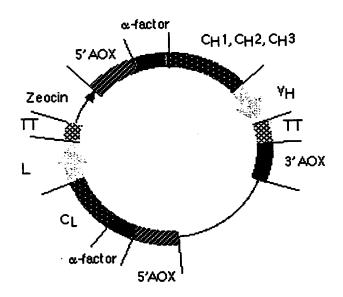
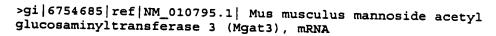


FIG. 23

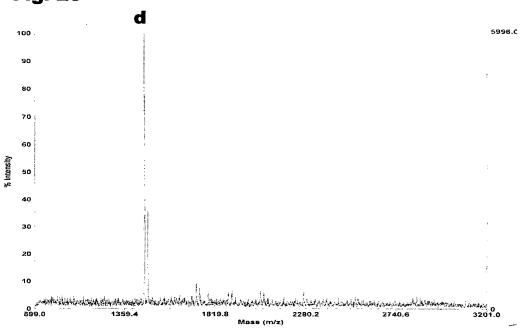
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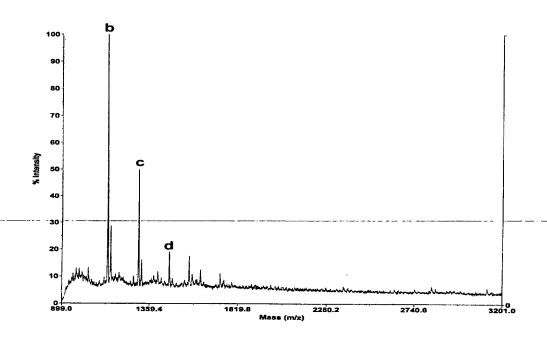


CAGCTTCTTCTGGAACAATGCCCCTGTCACTCCCCAGGCCAGTCCGGAGCCGGGTGGCCCGACCTATTG CGGACACCCCTCTACTCCCACTCTCCCCTGCTCCAGCCACTGTCCCCGAGCAAGGCCACAGAGGAACTGC ACCGGGTGGACTTCGTGTTGCCGGAGGACACCACGGAGTATTTTGTGCGCACCAAAGCTGGTGTGTG $\tt CTTCAAACCAGGATGCTGGAGAAACCTTCGCCAGGGCGGACAGAGGAGAAGCCCGAAGTGTCT$ GAGGGCTCCTCAGCCCGGGGACCTGCTCGGAGGCCCATGAGGCACGTGTTGAGTACGCGGGAGCGCCTGG GGTGCCCACGGTGGTGCAGTATTCCAACCTGCCCACCAAGGAACGCCTGGTACCCAGGGAGGTACCGAGG CGGGTTATCAACGCCATCAACATCAACCACGAGTTCGACCTGCTGGATGTGCGCTTCCATGAGCTGGGAG ATGTTGTGGACGCCTTCGTGGTCTGAATCTAATTTCACCGCCTACGGGGAGCCTCGGCCGCTCAAGTT ${\tt CCGAGAGATGCTGACCATTCGAGTACATCCGCCACAAGGTGCTCTATGTCTTCCTGGACCAT}$ TTCCCACCTGGTGGCCGTCAGGACGGCTGGATTGCGGATGACTACCTGCGCACCTTCCTCACCCAGGATG GCGTCTCCCGCCTGCGCAACCTGCGGCCCGATGACGTCTTTATCATCGACGATGCGGACGAGATCCCTGC GCGTGATGGTGTGCTGTACCTCAAACTCTACGATGGCTGGACAGAGCCCTTCGCCTTCCACATGCGGAAG TCCCTGTATGGTTTCTTCTGGAAGCAGCCGGGCACACTGGAGGTGGTGTCAGGCTGCACCATGGACATGC TGCAGGCCGTGTATGGGCTGGATGGCATCCGCCTGCGCCGCCAGTACTACACCATGCCCAACTTCCG GCAGTATGAGAACCGCACCGGCCACATCCTAGTGCAGTGGTCTCTCGGCAGCCCCCTGCACTTCGCGGGC TGGCATTGCTCCTGGTGCTTCACACCCGAGGGCATCTACTTTAAACTCGTGTCAGCCCAGAATGGCGACT TCCCCCGCTGGGGTGACTATGAGGACAAGAGGGACCTCAATTACATCCGCAGCTTGATCCGCACTGGGGG ATGGTTCGACGGAACGCAGCAGGAGTACCCTCCTGCGGACCCCAGTGAGCACATGTATGCTCCTAAATAC CTGCTCAAGAACTATGACCAGTTCCGCTACTTGCTGGAAAATCCCTACCGGGAGCCCAAGAGCACTGTAG AGGGTGGGCGCCAGAACCAGGGCTCAGATGGAAGGCCATCTGCTGTCAGGGGCAAGTTGGATACAGTGGA GGGCTAG

>gi|2117717|pir||JC4362 beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase (EC 2.4.1.144) III - mouse
MRRYKLFLMFCMAGLCLISFLHFFKTLSYVTFPRELASLSPNLISSFFWNNAPVTPQASPEPGDPDLLRT
PLYSHSPLLQPLSPSKATEELHRVDFVLPEDTTEYFVRTKAGGVCFKPGTRMLEKPSPGRTEEKTEVSEG
SSARGPARPMRHVLSSRERLGSRGTRRKWVECVCLPGWHGPSCGVPTVVQYSNLPTKERLVPREVPRRV
INAININHEFDLLDVRFHELGDVVDAFVVCDSNFTAYGEPRPLKFREMLTNGTFEYIRHKVLYVFLDHFP
PGGRQDGWIADDYLRTFLTQDGVSRLRNLRPDDVFIIDDADEIPARDGVLFLKLYDGWTEPFAFHMRKSL
YGFFWKQPGTLEVVSGCTMDMLQAVYGLDGIRLRRRQYYTMPNFRQYENRTGHILVQWSLGSPLHFAGWH
CSWCFTPEGIYFKLVSAQNGDFPRWGDYEDKRDLNYIRSLIRTGGWFDGTQQEYPPADPSEHMYAPKYLL
KNYDQFRYLLENPYREPKSTVEGGRQNQGSDGRSSAVRGKLDTAEG

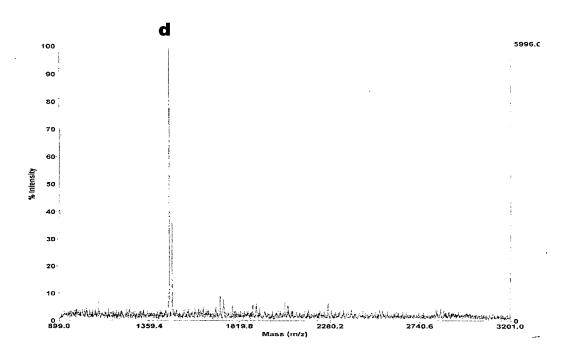


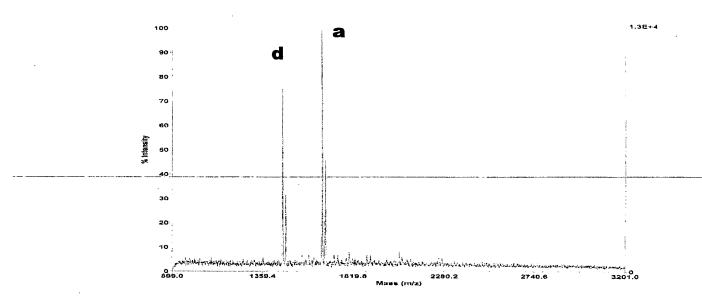




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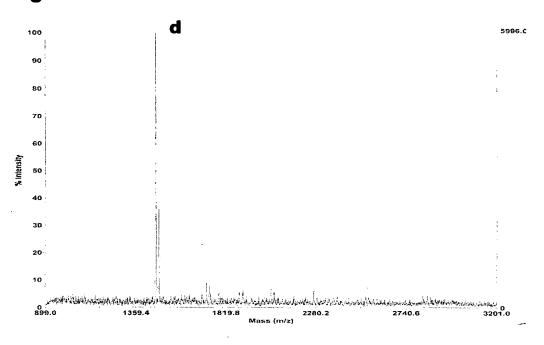
Fig. 26

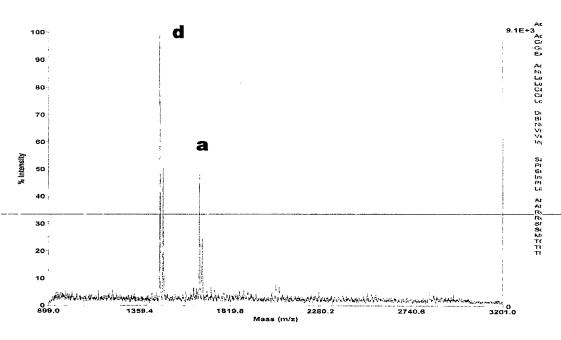




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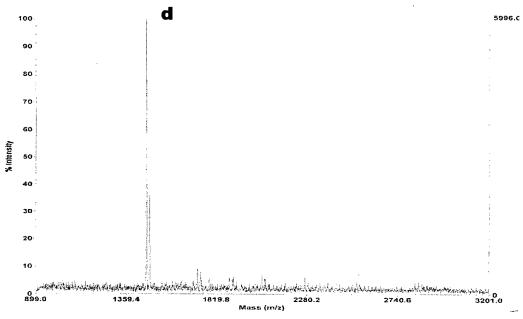
Fig. 27

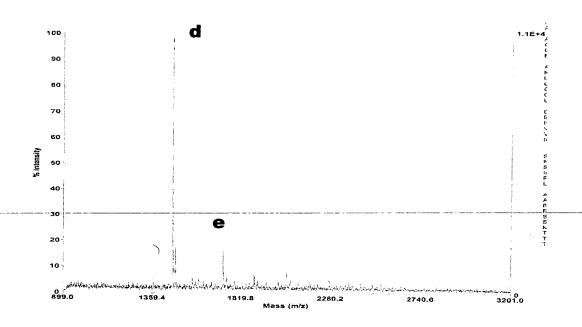




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Fig. 29

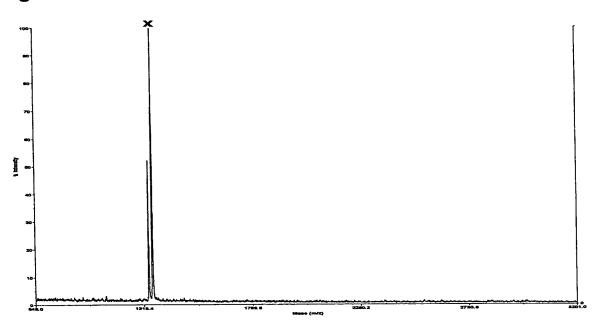


Fig. 30

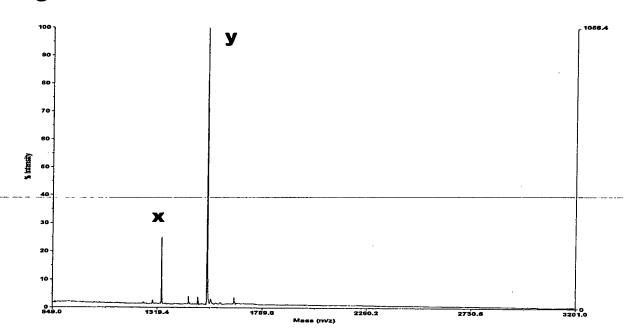


Fig. 31

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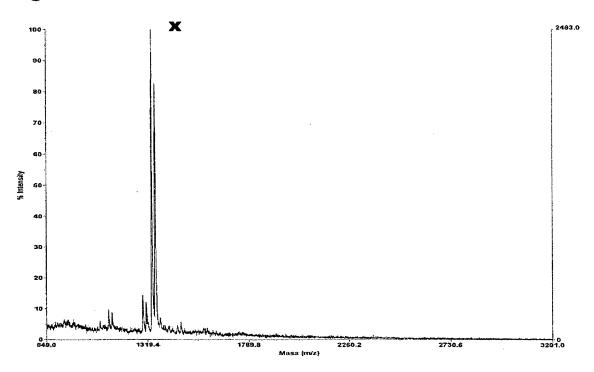
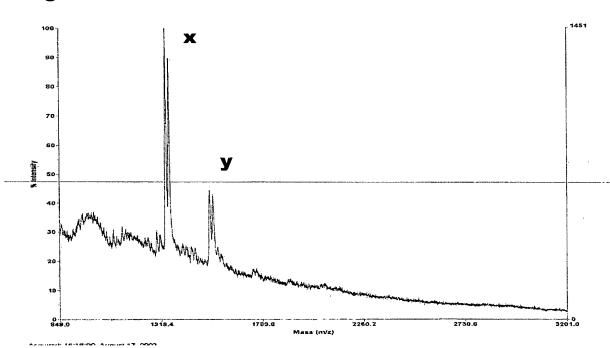


Fig. 32



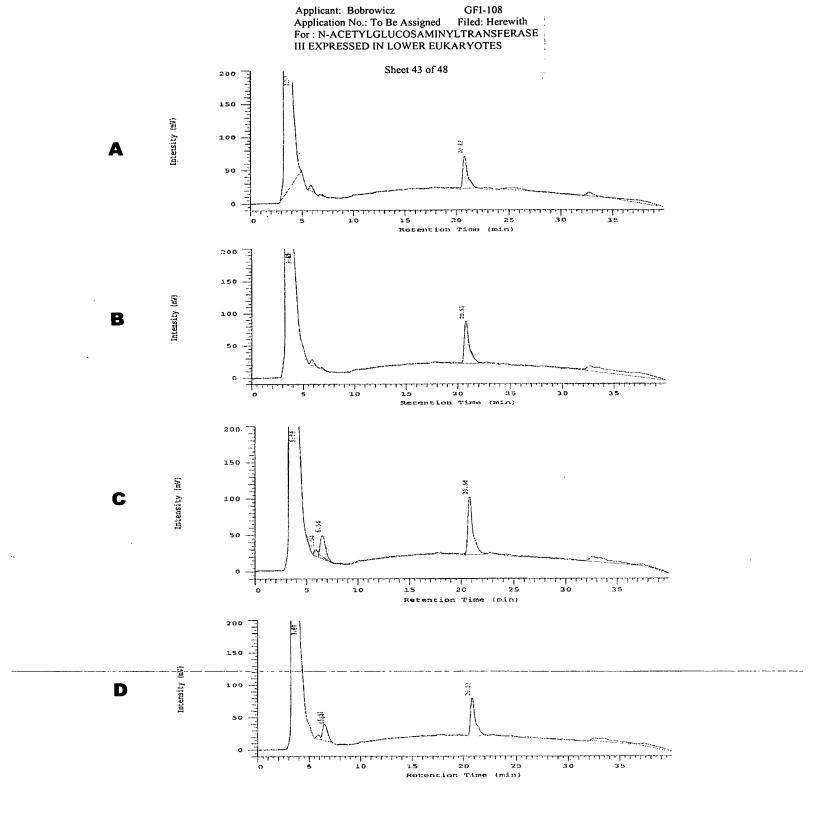


Fig. 33

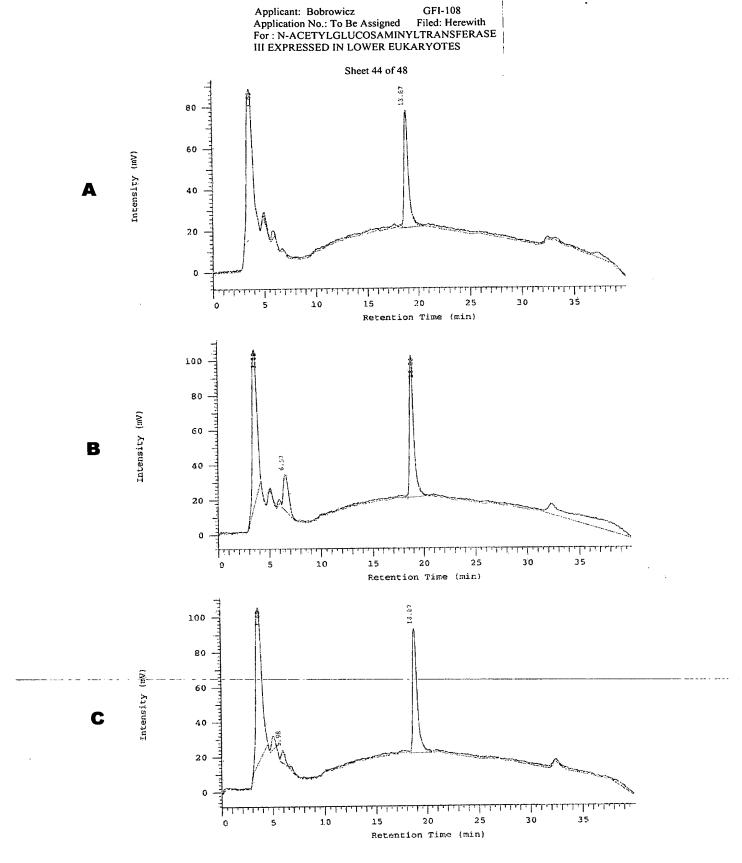
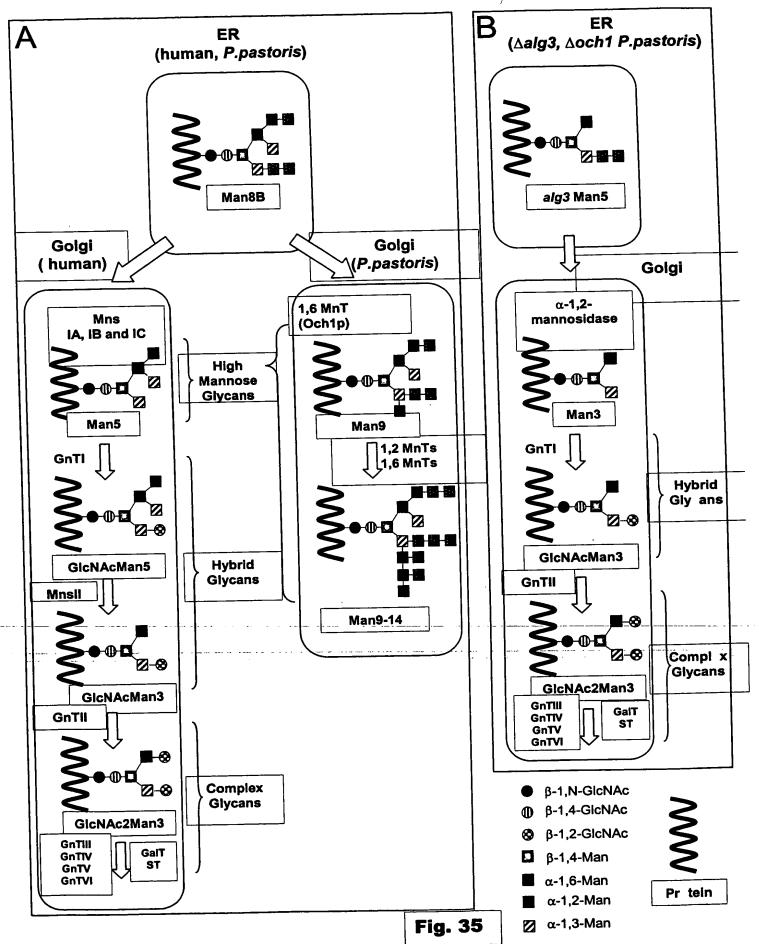


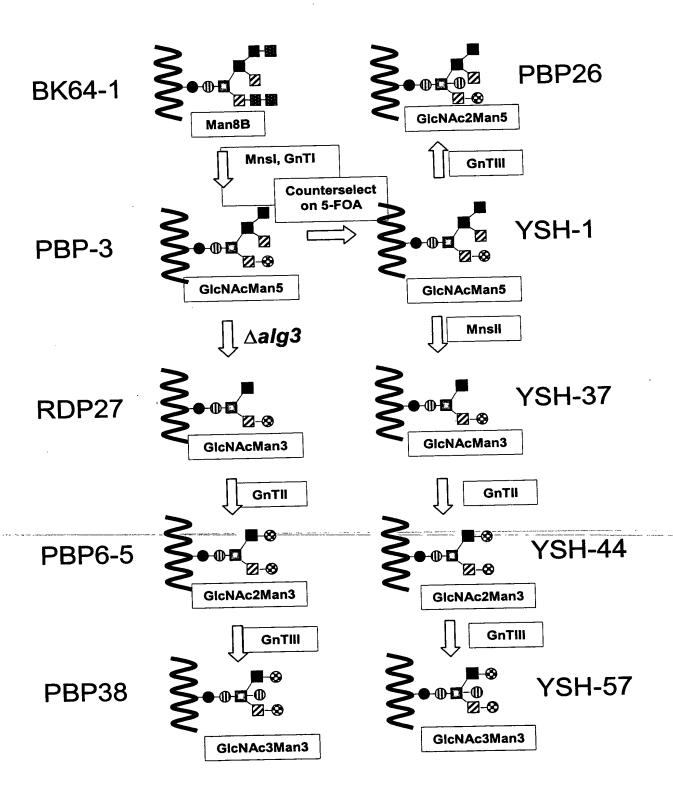
Fig. 34

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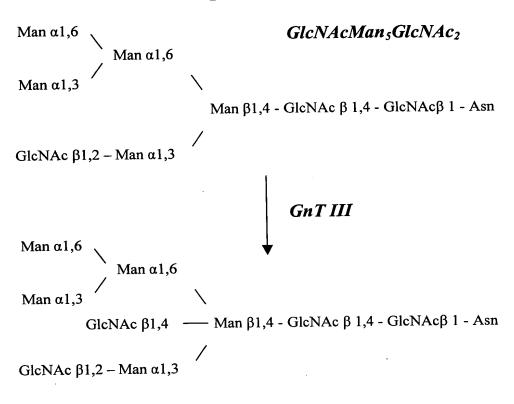
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Fig. 36



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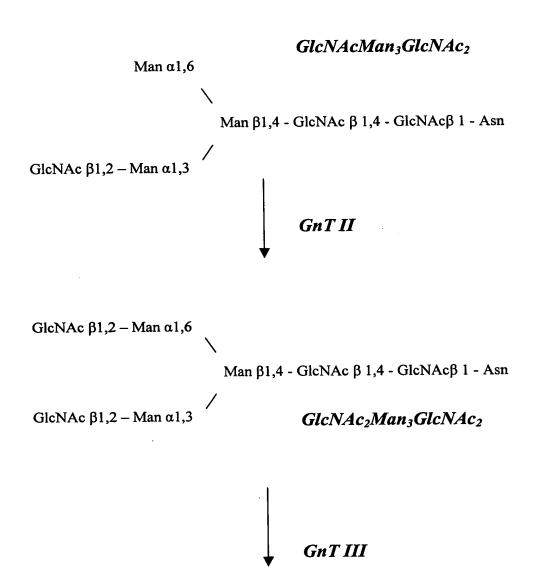
Fig. 37



GlcNAc2Man5GlcNAc2

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Fig. 38



GlcNAc
$$\beta$$
1,2 – Man α 1,6
GlcNAc β 1,4 – Man β 1,4 - GlcNAc β 1,4 - GlcNAc β 1 - Asn GlcNAc β 1,2 – Man α 1,3

 $GlcNAc_3Man_3GlcNAc_2$